HEAD SMUT OF SORGHUM AND MAIZE

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GENERAL CHARACTERISTICS OF THE DISEASE

DISTRIBUTION

In the agriculture of western Kansas and Texas and similar parts of the Great Plains area various sorghum varieties have recently attained considerable importance as a dry-land crop in the farming operations which are developing in the sections formerly devoted to cattle ranges. This fact, together with the importance of broom corn in some sections, has led to an investigation of the diseases of the sorghum crop by the Office of Cereal Investigations of the Bureau of Plant Industry.

The study of the head smut has an added importance from the fact that it occurs on maize (Indian corn) and has been reported by McAlpine (1910, p. 290) [1] as serious on that crop in Australia, and by Evans (1911) and Mundy (1910, p. 1) in South Africa (Pl. XXXI). It has been found on maize in some abundance in this country (Norton, 1895; Hitchcock and Norton, 1896, p. 198), although the writer, in rather extensive observations, has never seen such a case; nor has it been recently reported.

The parasite is widely distributed in sorghum-growing regions throughout the world, and in some sections, chiefly tropical or subtropical, it is very destructive. Munerati (1910, p. 718) has found it abundant on *Sorghum halepensis*, and it has also been reported from Italy by Passerini (1877, p. 236), Mottareale (1903, p. 3), and Cugini (1891, p. 83); from India by Cooke (1876, p. 115) and Barber (1904); from Egypt by Kühn (1878, p. 10); from German East Africa by Busse (1904, p. 378); and from Japan by Hori (1907, p. 163). According to Hennings (1896, p. 119), it occurs in North and East Africa, Madagascar, and East India, as well as in Central and South Europe. While it has been reported from Iowa, Illinois, Kansas, Minnesota, Mississippi, Nebraska, New Jersey, Ohio, and Texas, according to Clinton (1904, p. 393), it is fortunately still quite rare in this country. Clinton states that it was probably introduced into the United States with importations of sorghum seed from Europe. This seems quite possible in considering Kellerman and Swingle's (1890, p. 159) original note on its occurrence in this country, where it is noted that it first occurred in New Jersey on Amber sorgo (sweet sorghum). In Kansas it was first noticed on "Red Liberian" (sumac)

[1] Citations to literature in parentheses refer to "Literature cited," p. 369-371.

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sorgo (Failyer and Willard, 1890, p. 145), which would suggest Africa as its source.

There appear to be three distinct forms of smut (Pl. XXXII, fig. 1) affecting the sorghum crop in America (Potter, 1912): *Sphacelotheca cruenta* (Kühn), *Sphacelotheca sorghi* (Link) Clint., and *Sorosporium reilianum* (Kühn) McAlp., the head smut (Pl. XXXII, fig 2). Of these the last-named alone has consistently resisted efforts to prevent its spread, though all known methods for the prevention of cereal smuts have been tried. The serious occurrence of the disease has been observed to be confined at present to the Texas Panhandle. For this reason the investigations, begun in 1907 by Dr. E. M. Freeman and continued after 1909 by the writer,[1] have been carried out chiefly at Amarillo, Tex., with plantings at other points for comparison. This work has been supplemented by studies in the greenhouse and laboratory at Washington, D. C.

SYNONYMY

The head smut of sorghum was first noted by Julius Kühn (1875), who described it from a specimen sent to him from Egypt by Dr. Reil in 1868.[2] The mistake he made in describing the spores as smooth was repeated by Passerini (1876) when he described the form of maize. The echinulations are often obscure, however, unless the spores are quite mature and dry. Brefeld (1883, p. 94) describes them as almost smooth.

Saccardo (1876) and de Toni (1888) described this smut as showing an aggregation of spores suggestive of Sorosporium, as did also Norton (1896, p. 233). Busse (1904, p. 381) suggests in this connection, as Brefeld (1883, p. 171) did earlier, that possibly the genus Sorosporium should not be retained. Busse notes and figures the characteristic spore aggregates, but states that this smut is intermediate in this respect between Ustilago and Sorosporium. According to Dietel (1900, p. 7), the two genera are not sharply distinguishable. Although the spores are rather loosely bound together in this species, McAlpine (1910a, p. 181) has recently placed it in the genus Sorosporium. Under the present artificial system necessitated by a lack of adequate knowledge of the natural relationships

[1] The author wishes to acknowledge the advice and assistance of Mr. E. C. Johnson, who was in charge of the cereal-disease work from 1908 to 1912, inclusive, during which time most of the work here presented was done. Considerable assistance has also been given by various officials at the stations where the work was performed, among whom Dr. E. M. Freeman should be especially mentioned.

[2] "*Ustilago Reiliana* Kühn in litt. U. sporis laevibus, subglobosis, crassiusculis (10, 4 Mikr. inter et 13, 3 Mikr. diamet. variantib.) semipellucidis, brunneis; paniculam totam contractam et obvolutam et abortivam corrumpens. Crescit in Sorgho vulgari." Rabenhorst's Fungi Europaei Exsiccati, No. 1998.

The name given by Kühn is still retained by European mycologists. Its synonymy follows:

Ustilago reiliana Kühn, 1875, *in* Rabenh., Fungi Europ. Exs., ed. nova, s. 2, cent. 20, no. 1998.

Ustilago reiliana, forma *zeae*, Pass., 1876, *in* Rabenh., Fungi Europ. Exs., ed. nova, s. 2, cent. 1 (resp. cent. 21), no. 2096.

Ustilago pulveracea Cooke, 1876, *in* Grevillea, v. 4, no. 31, p. 115, pl. 63.

Cintractia reiliana Clint., 1900, Ill. Agr. Exp. Sta. Bul. 57, p. 346.

Ustilago (*Cintractia*) *reiliana* forma *foliicola* Kellerm., 1900, *in* Ohio Nat., v. 1, no. 1, p. 9, pl. 2.

Sphacelotheca reiliana Clint., 1902, *in* Jour. Mycol., v. 8, no. 63, p. 141.

Sorosporium reilianum McAlp., 1910, Smuts of Austral., p. 181.

in this group this classification seems proper in view of his illustration (pl. 30, fig. 37) and of our Plate XXXIII. From these it is evident that the spores, as they occur aggregated into irregular groups, are so formed in the sorus, for the spore balls are found before the spores are mature or even before the latter are differentiated—i. e., while the fungus is still in the hyphal stage.[1]

GROWTH IN ARTIFICIAL CULTURES

The recent work of Appel and Riehm (1911, p. 346, pl. 4[2]) has again emphasized the fact, first established by Brefeld, that the smuts can be cultivated on artificial media in their saprophytic stages. Similar work with this organism has been found difficult on account of trouble in collecting spore material free from contamination and thoroughly germinable. Indeed, the writer has rarely succeeded in getting over 15 per cent of the spores to germinate. The large, open sorus, moist with the saccharin juices of the host, gathers yeasts, molds, and bacteria, which are very troublesome, particularly in liquid cultures. These were attempted repeatedly in several different seasons and at various times of the year, but with only slight and irregular germinations, no matter what the age, source, or condition of the spores. Cane-sugar solutions were largely used, as well as distilled water, rain water, tap water, soil decoctions, sorghum sap, beef bouillon, decoctions of carrots and of prunes, Uschinsky's solution, and Cohn's solution, the last named being also tried in the modified form used by Hitchcock and Norton (1896, p. 200) in their work with this smut. The temperatures were not controlled or recorded in most cases.

With solid media, however, the isolation of the spores found germinating was accomplished by transplanting them with glass hairs under the binocular microscope to sterile poured plates, where their development into conidial colonies was watched under the microscope. Plates seeded thinly enough to contain few contaminations would so seldom show any germinating spores that transplanting from a thickly seeded plate proved to be the only practicable method of isolating, since the head-smut colonies developed so slowly at ordinary temperatures (over a week was required after germination for the colony to become visible to the naked eye) that the plates would be obscured by other organisms long before the smut could be isolated in the usual way. Moreover, the method employed made it certain that the conidia thus obtained in pure culture were not those of some contaminating yeast. It should be said, however, that since this was done it has been found that the yeast and bac-

[1] The character of the sorus, particularly in the decided deformity of the whole inflorescence, also seems more closely similar to several of the species of Sorosporium than to any of Sphacelotheca as described by Clinton (1904, p. 383–395). Although the observations here presented do not appear to be in accord with the classification given this form in Clinton's monograph, the writer is much indebted to Dr. Clinton for helpful criticism.

[2] Erroneously marked "plate 3."

terial contaminations (not the molds) can be almost entirely eliminated without injury to the spores by treating with copper sulphate (see p. 356–357).

The isolation of the organism gave excellent opportunity for a closer study of its relation to various media and temperatures. Plate XXXIV, fig. 1, shows its growth in about six weeks from transfer on carrot agar at 20° to 23°, 30°, 35°, and 40° C., respectively. At 40° there is no growth. At 35° the growth is very slight, light brown in color, and much attenuated. A culture at 32.5° C. grew poorly, and those at higher temperatures were eventually killed, for they did not grow on being removed from the incubator. The rapid development at 30° indicates that this is very near the optimum temperature for the organism, and this is borne out by the studies of germination given in Table I.

TABLE I.—*Germination of spores of the head-smut organism at various temperatures*

Serial No.	Date of test.	Temperature.	Duration of test.	Germination.
	1912.	°C.	*Days.*	*Per cent.*
1	Dec. 16	29–31	3	6.0
2	...do.....	[a]20–21	3	2.0
3	...do.....	16–20	2	.2
4	...do.....	17	3	.2±
5	...do.....	14.5	3	0
6	...do.....	12	3	0
7	...do.....	8.5	3	0
8	...do.....	7.5	3	0
9	...do.....	4	3	0
10	...do.....	1	3	0
	1913.			
11	Jan. 8	40	3	0
12	...do.....	37.5	3	0
13	...do.....	35	3	0
14	...do.....	32.5	3	1.5
15	...do.....	30	3	7.9
16	...do.....	[a]23–25	3	3.0
17	...do.....	[a]20–23	3	1.0
18	...do.....	18–20	3	2.0
19	...do.....	20–23	3	5.0
20	Mar. 18	27	8	4.0
21	...do.....	17	8	.4
22	...do.....	9	8	0
23	...do.....	23	8	2.0+
24	Mar. 19	27	7	13.1
25	...do.....	17	7	1.0

[a] All but these were incubated in the dark.

These germinations were made in carrot-agar plates with material collected at Amarillo, Tex., in September, 1911, from Red Amber sorgo, except the last two, which were from kafir grown in 1912. From Nos. 11 to 19, inclusive, the number of spores counted in each case was 200; for the rest of the tests the count was not recorded except as follows: No. 20, 1,000; No. 23, 500; No. 24, 541; and No. 25, 818.

In respect to its optimum temperature, then, the head smut is quite unlike those smuts which infect chiefly from seed-borne spores.[1] It is, on the other hand, closely similar to those infecting intraseminally—i. e., the loose smuts of barley and wheat (Appel and Riehm, 1911, p. 364)—and also seems to resemble corn smut, *Ustilago zeae* (Beckm.) Ung., which, while infecting extraseminally, has a late period of infection and shows a more or less localized development. Preliminary observations on corn smut indicating a similar relatively high optimum temperature were made at the same time as Nos. 11 to 19, inclusive, in Table I; and it is this analogy, rather than that with the loose smuts, which has been supported by the evidence of inoculations and other experiments, presented later.

The fact that the head smut is indigenous to a host from subtropical climates should also be pointed out in this connection. At low temperatures, however, the organism can not be said to be injured, although it grows very slowly, if at all. Even severe freezing does not kill it. Both the spores and conidia have been frozen at St. Paul, Minn., at outdoor temperatures which reached a minimum of $-26°$ C., in both a wet and dry condition, and some were still found to be viable, though frozen for over three weeks. Similar tests at Amarillo, Tex., and at Washington, D. C., were generally confirmatory of these results, although much weathering sometimes appeared to destroy viability.

The writer has not found the spores readily germinable after several years, as did Brefeld (1883, p. 95). Furthermore, the conidia have not survived periods of drying, lasting from four to eight months at ordinary summer temperatures. The method used for determining the latter was to smear some cover glasses with conidia from carrot-agar culture and leave in a Petri dish or culture tube for the period mentioned before transferring to a culture medium for test of viability.

The organism has been found to develop well on malt extract and beerwort agars—perhaps even better than on carrot agar. A synthetic dextrose agar is also favorable. Plate XXXIV, figs. 2 and 3, shows the characteristic, rugose conidial growth. Carrot agar gives a more rapid growth, but the darkened central area of the culture shown in Plate XXXIV, fig. 3, is becoming brown. This may be caused by differences in drying or by the influence of contaminations near it in the plate. A malt extract prepared from germinated Amber sorgo seed was tried, but did not prove to be as favorable a medium as the others. On a 3 per cent cane-sugar agar the growth was scant. Gelatin is liquefied readily. While the organism grows well in 1 per cent peptonized (1 per cent of peptone) solutions of saccharose, lactose, levulose, dextrose, and maltose,

[1] See Herzberg (1895, p. 23) on *Ustilago avenae*. Dr. H. B. Humphrey, at present pathologist in the Office of Cereal Investigations, has found in unpublished experiments that *Tilletia tritici* has an optimum temperature of very close to 20° C.

it does not ferment any of them. Spores, or decidedly sporelike bodies [1] (Pl. XXXIV, fig. 4), are frequently formed in liquid cultures, which then show the brown color characteristic of the resting stage. These may also be found occasionally in agar cultures. They are usually undersized (7.5 to 12μ) and show only traces of echinulations. Their germination has not been observed. In the upper part of the figure (Pl. XXXIV, fig. 4) are shown some of these artifically grown chlamydospores (on the left) with natural spores (on the right) for comparison. Below are shown chains of spores and examples of peculiar formations which are suggestive of the involution forms in many bacteria.

FLORAL ALTERATIONS

A peculiar reaction between this parasite and the host manifests itself by a vegetative stimulus to the host, not only in the vegetative parts but also in the inflorescence.

The parasite of head smut does not always develop a sorus on an infected culm, but frequently causes a floral sterility (Pl. XXXV, fig. 1) which develops at times into a peculiar proliferation of the panicle (Pl. XXXV, fig. 2). This phenomenon, in the tassels of maize, has already been noted and figured by Hitchcock and Norton (1896, p. 199). In extreme cases of this sort in sorghum (Pl. XXXV, fig. 2) the ovary and stamens entirely disappear and the growth takes the form of a complete individuation in the place of each flower; a tiny culm, with leaves, nodes, and rudimentary panicle, shoots up from the head almost as if in an effort to escape the parasite. The hyphæ of the latter were found in one instance to have penetrated the tissues of the phyllomorphic or almost phytomorphic flower (Pl. XXXVI). They are distinctly shown in the illustration as darkly stained threads in the upper part of the panicle and in the bud at its base. In some of the parenchymatous tissue the nuclei are abnormal and have taken the stain like the hyphæ. A number of other flowers less strongly proliferated were examined and found to contain no hyphæ. It may be concluded from this that the change is probably caused by alterations in nutrition processes, especially since a somewhat similar though less pronounced phyllomorphism has been observed in districts where the head smut does not occur, as at Arlington, Va. (Kusano, 1911).

Where the smut occurs commonly, however, this proliferation of the inflorescence is very characteristic and furnishes a more ready means of distinguishing the infected plants than the presence of the sori themselves. Indeed, of 125 plants of Red Amber sorgo examined in three different seasons (1910, 1911, and 1912), mostly at Amarillo, only two

[1] Brefeld (1883, p. 158) obtained the spores of *Tilletia tritici* in artificial culture and Busse (1904, p. 375) has done so with another sorghum smut, *Ustilago cruenta* Kühn. He did not culture the head smut, doubtless because of the interference of contaminations which he mentions (p. 377). Grüss (1902, p. 219) has described spore formation in *U. zeae* in cultures. Herzberg (1895, p. 7) does not consider them analogous to those formed on the host, although he germinated some of them in the case of *U. tritici*.

were found to be wholly smutted—i. e., producing spores in every head. Infected plants of this variety almost always have some normal culms, although the number of these varies greatly with the season. Of the 125 plants examined, 64, or more than 50 per cent, produced one or more culms with normal panicles. An infected culm may bear a normal head, but this is rare. Usually such a culm bears no seed, and there is almost always some degree of abnormality in evidence, the glumes becoming elongated and either decolorized or of a greenish hue.

INFECTION OF NODAL BRANCHES

Along with these floral changes there usually occurs an abnormal tendency to branch. Indeed, the development of the buds, which occur alternately on opposite sides of the culm at each node, much as in other Gramineae (Hackel, 1887, p. 3), is often the only positive evidence of the infection, since the resulting branches usually bear sori. This phenomenon has led Busse (1904, p. 386–392)[1] to consider the infection of a branch to take place from hyphæ within the node, growing up through the tissue of the sheath at the time the bud begins to develop, and he evidently concludes (p. 391) that these nodal buds are not infected until they begin to grow out into branches. The histological data given in support of his view seem inadequate to establish, beyond a question, his identification of smut hyphæ in the lesions which sometimes occur in the sheath over the swollen buds. The present investigation has shown, too, that these buds become infected without reference to their development into branches and that there is a peculiar regularity about the infection even when some of the branches are missed.

Forty culms from 15 infected plants of Red Amber sorgo (S. P. I. No. 17548) grown at Amarillo were dissected and studied for the occurrence of the parasite in the nodal buds, and the results are summarized in figure 1. The material was killed and fixed with aceto-alcohol (Carnoy's fluid), a mixture of one-third of glacial acetic acid and two-thirds of commercial alcohol, for periods varying from 2 to 24 hours. It was then rinsed in two or three changes of 70 per cent alcohol and kept in this until embedded in paraffin in the laboratory at Washington, D. C. All the buds from a single culm were prepared and kept together in one vial and were distinguished from each other by cutting them into different shapes, which were sketched into a record showing their position on the culm.

The oft-recurring difficulty in definitely differentiating between the host and parasite by staining methods was encountered in this work. After experimentation it was found that this organism is Gram-positive under most conditions, and with a counterstain of eosin in clove oil a very

[1] Busse (1904, p. 391) says, "Ich nehme an, dass die Infektion nicht direkt, sondern auf dem Umwege über die mit dem Stengel organische verbundene Hauptsprossscheide zu stande kommt." *See also* his Pl. V, figs. 15, 18, 18c, and 19.

sharp contrast was obtained. This proved to be a quick, convenient method, and the stain is fairly permanent if the clove oil is carefully washed off with xylol before mounting in balsam.

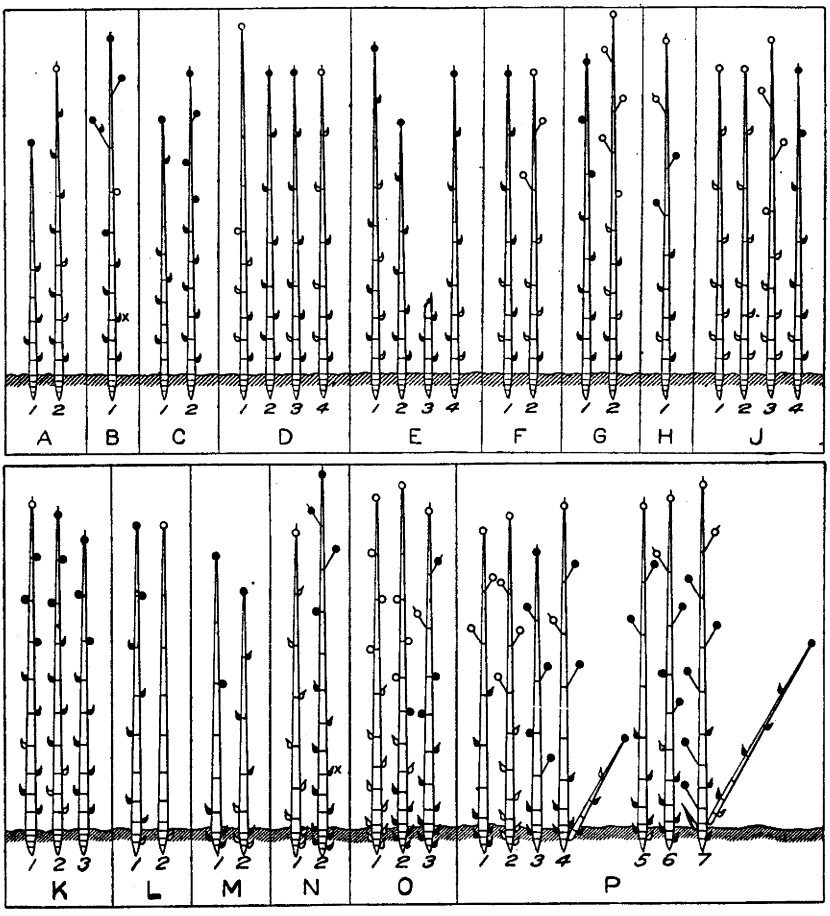

FIG. 1.—Diagrammatic representation of the occurrence of infection in the nodal buds or branches of several sorghum plants.

In figure 1 each plant is designated by a letter and its culms by numerals. The culms are represented with nodes and with branches where they occurred, but without leaves, sheaths, or roots. The growth at each node is represented as follows: A bud which has developed a panicle, either directly evident, as in the main inflorescence, or rudimentary and discovered in dissecting, is represented by a circle, while buds developed to a lesser degree are represented by a subovate symbol. Those showing spore development or, upon microscopic examination, the presence of the hyphæ of the parasite, are shown in solid black, while those which were normal are in outline. In cases where the panicle was not completely parasitized or where the inflorescence, while showing no spore formation, was wholly or partially sterile, the culm is represented as extending through it, the presence or absence of spore formation being indicated as above. When no growth is represented at a node, it signifies that the bud was lost in handling or that for some other reason it was not examined.

All the plants represented in figure 1, except O, were dissected in the early autumn of 1910 at Amarillo. Plant O was one of a number prepared in 1911. In plants A to K, inclusive, no buds were taken from below the surface of the ground. In all cases, however, the exact position of the ground line was not recorded, but has been assumed. The buds on the suckers shown in plant P were not necessarily situated as shown, since they were too small to differentiate by the method used. Culm P3 also bore a sucker at the first node, on which three buds were infected and three apparently undiseased, the apical bud being lost.

FIG. 2.—Diagram of Plate XXXVII, figure 1, showing the position of the hyphæ.

An examination of the diagrams reveals the fact that most of the culms were but partially infected. A particularly noticeable feature is that when only a few of the buds were missed by the parasite they occurred neither at consecutive nodes nor yet irregularly, but almost without exception included only such as were on the same side of the culm. This is well illustrated in culms A2, D3, E1, E4, F1, J4, and L1. In the same way, if only a few of the buds were involved in the infection, they, too, were usually on the same side of the culm and at the base of the plant, as seen in culms D1, D4, and F2. The basal portion sometimes escaped (as in culms K2 and M2), and occasionally the top grew away from the

parasite (as in culms O2, O3, P1, P5, P6, and P7), though usually remaining sterile. Thus, the plant is seen to have been infected only in such of the buds as were developed from a definite section of the original meristem. The few irregularities (culms G2, H1, K1, O2, and P6) can not be said necessarily to conflict with this interpretation, but were probably the result of unusual developments, such as a double infection, or, perhaps, of errors in technique or records in repeatedly handling these 300 or more buds. It seems certain that the dominance of cases showing regularity of infection can not be due to error.

Plate XXXVII illustrates the appearance of the hyphæ in two of these nodal buds. The two buds in question are marked by a cross in text figure 1. In Plate XXXVII, figure 1, the host tissue was stained more deeply than in the other, and the hyphæ, which are intercellular, do not show as well, particularly those not exactly in focus. Text figure 2 will assist in locating such as are discernible in Plate XXXVII. It should be noticed that in this section the hyphæ are seen mostly in the tissues on the left, while in the other nearly all of them are on the right. Such an arrangement doubtless occurred in the buds from which such infections developed as are shown in culms A2, D4, E1, F1, etc.

It is apparent that no assumption of the occurrence of the primary infection at or near the maturity of the host can explain the regularities of the infection phenomena usually found in these buds without also assuming an improbable spread of the infection in the mature tissues of the host. The nodal branches were evidently infected early, when the buds formed, if at all. As Brefeld (1895, p. 47, 84) observed in connection with his work on infection with *Ustilago cruenta*, the sorghum plant grows very slowly at first for a period of about four weeks or more. It was during this time, then, while the meristem, at least in each culm, was confined to a comparatively small compass, that the spread of the infection must have proceeded in such a way as to determine its later development in these plants.

LIFE HISTORY OF THE PARASITE

PREVIOUS WORK

That the head smut infects its host in the early seedling stage has been the general assumption as to its life history, although the results of inoculations performed by investigators would seem to have given doubtful support to the idea. Brefeld (1883, p. 94) states that Kühn, who named this parasite, obtained a double, artificial infection with this smut and *Ustilago cruenta*. Passerini (1877, p. 236) says he was able to reproduce the head smut on maize, but not on sorghum. W. A. Kellerman (1891, p. 98, 101) produced slight infection in greenhouse and field experiments by inoculating the seed. Later (1900a, p. 9)[1] he

[1] See "Literature cited" for notes published in 1898; with K. F. Kellerman in 1899; and with O. E. Jennings, reporting further negative results, in 1902.

produced it also on maize and described the form *foliicola*. While he states (1900, p. 18) that infection from seed-borne spores takes place and that, therefore, seed treatment with fungicides is of value, he had, like Passerini, produced the disease, in the field, only on maize and in very small quantities. Clinton (1900, p. 347) also failed to produce any infection by inoculations of the seed and young plants. Hori (1907, p. 163, 166) reports entirely negative results from inoculations, but claims that a hot-water treatment has been shown to prevent the disease. McAlpine (1910, p. 296) produced infection in a single maize plant by seed inoculation and on this basis recommended seed treatment with copper-sulphate solution as a preventive. Johnston (1910 or 1910a, p. 44) has also recommended seed treatments, and this Australian idea has been copied by Mundy (1910, p. 4) in South Africa.

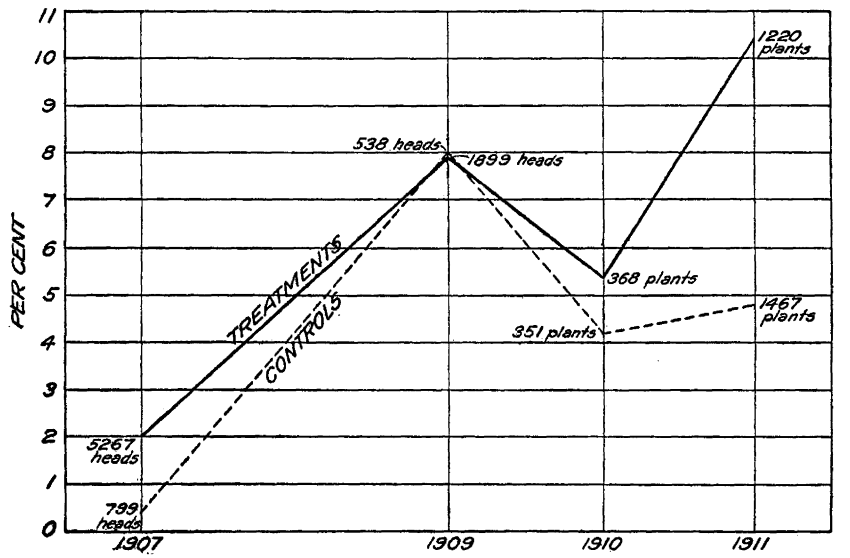

FIG. 3.—Curves summarizing for different years the percentages of infection in plantings of sorgo after all hot-water treatments and in control plantings.

The early inoculation experiments of the Office of Cereal Investigations, involving about a thousand plants of different varieties (including kafir and sorgo) in the field at Amarillo, gave results similar to those cited above—i. e., little or no infection resulted from the presence of an abundance of spores on the seed.

SEED AND SPORE TREATMENTS

In full accord with the negative results of these inoculations our experiments have conclusively shown that the most severe treatments of the seed, though carefully performed, do not prevent the attack of the parasite. These treatments have involved some 35,000 or more plants, of which about two-thirds were in tests of thermal methods, the

rest of the tests being performed with fungicides. For the latter, formalin, copper sulphate, cresol, and potassium sulphid were tried. Kafir, broom corn, and sorgo were used, and of these the first two developed so little infection that the results were of no significance.

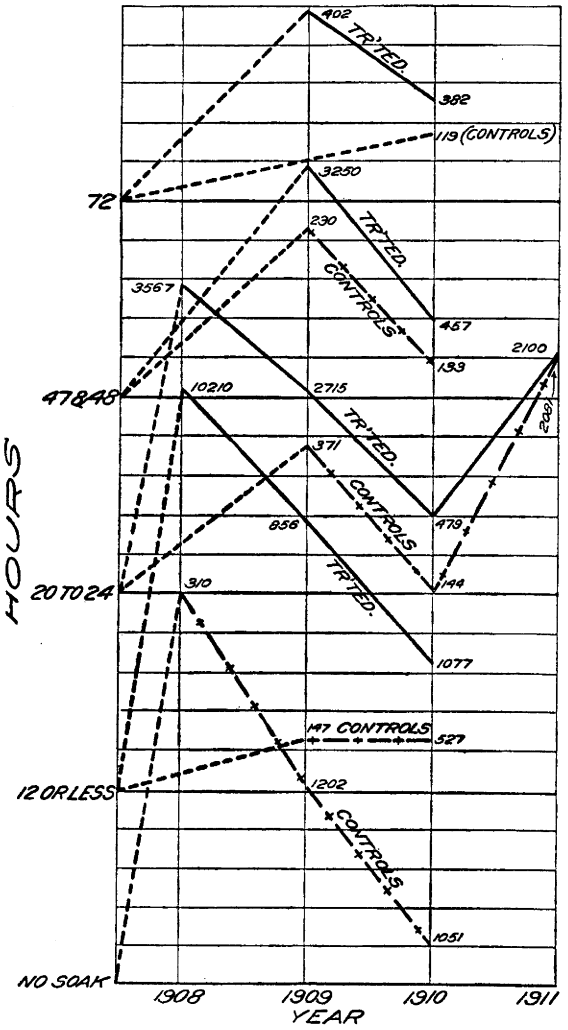

FIG. 4.—Curves summarizing for different years the percentages of infection: First, in plantings of Red Amber sorgo after modified hot-water treatments at all temperatures and of all durations, but after presoakings of various duration; and, second, in control plantings (not treated with hot water).

With the more susceptible sorgos (chiefly the Red Amber variety), however, quite heavy infections occurred in some seasons. The important features of the results are brought out in the summaries presented in figures 3 to 7, inclusive. The first and last of these figures present results obtained with several varieties of sorgo, the one being a summary of treatments performed with hot water without presoaking and the other a summary of the whole work on seed treatments, including both thermal and chemical methods. The three others (figs. 4, 5, and 6) show the results of modified hot-water treatments [1] of Red Amber sorgo (S. P. I. No. 17548) according to the three elements of the treatment: figure 4, according to the length of presoaking given the seed; figure 5, according to the duration of the hot-water treatment; and figure 6, according to its temperature.

[1] This method was originated by Jensen (1888). See Freeman and Johnson (1909) and Appel and Riehm (1911). Tepid water for presoaking was tried in a few of these treatments of sorghum, but without any difference in results.

In summarizing the results for constructing these curves, the duration of presoaking in the modified treatments and the duration and temperature of treatments have been approximated in several instances in order to bring all of them to intervals of 12 hours of presoaking, 5 minutes in duration of treatment, or 2 degrees in temperature. The results of treatments performed in 1909 and previously were recorded by counting heads, while subsequently they were recorded by noting the number of plants. These numbers are given at each point in the curves.

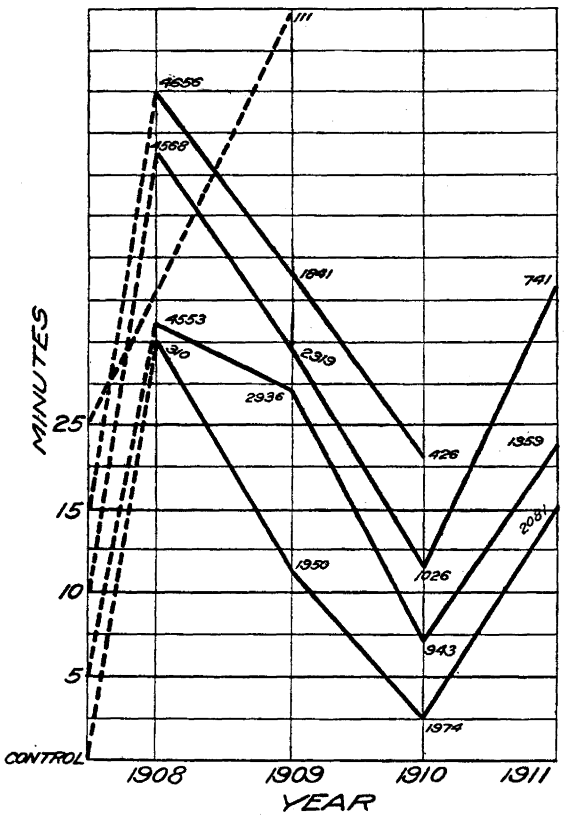

FIG. 5.—Curves summarizing for different years the percentages of infection in plantings of Red Amber sorgo after modified hot-water treatments at all temperatures and of all presoakings, but after treatments of various durations.

It is evident from the curves in all these illustrations not only that the treatments in no way reduced the amount of infection, but also that, regardless of treatment, the percentage of smutted plants occurring varied consistently with the season. Indeed, the curves in figures 4, 5, and 6 proved to be, with scarcely an exception,[1] so nearly alike for all the treatments that they could not well be drawn to the same coordinates. They are therefore separated, and each curve is continued by a broken line to the axis of the coordinates to which it is drawn, each interval therefrom representing 1 per cent of infection.

While it is true that infection by any phytopathogenic organism would vary with seasonal conditions regardless of the exact features of its life history, an added significance in these curves is found when it is noted that

[1] The only case in which these curves do not very nearly coincide is in the 54° C. treatments of 1909 (fig. 6). In this case there were but 151 heads on which to base the 1909 figure, this being so small that the result, which is characteristic of the irregular occurrence of the infection at Amarillo, is plainly dependent upon some peculiar minor factor, such as a variation in soil conditions, rather than upon the season. It is certainly not owing to the treatment of the seed.

the plantings at the Cereal Field Station at Amarillo were on new land both in 1907 and 1910. This station was established in 1907 and removed to another situation, also at Amarillo, at the latter date. In view of the fact that the presence of the organism has proved to be so salient a factor, as established by seed exchange and inoculation experiments, presented later, it would seem proper to attribute the light infection in 1907 and 1910 to the relative scarcity of the infective stage of the organism in the virgin soil. The large increase in 1908 was probably due to the proximity to the station of an old field which grew a rather badly smutted crop of sorgo each year. The decrease in 1909 was doubtless caused by drought, scarcely half of the crop being headed.

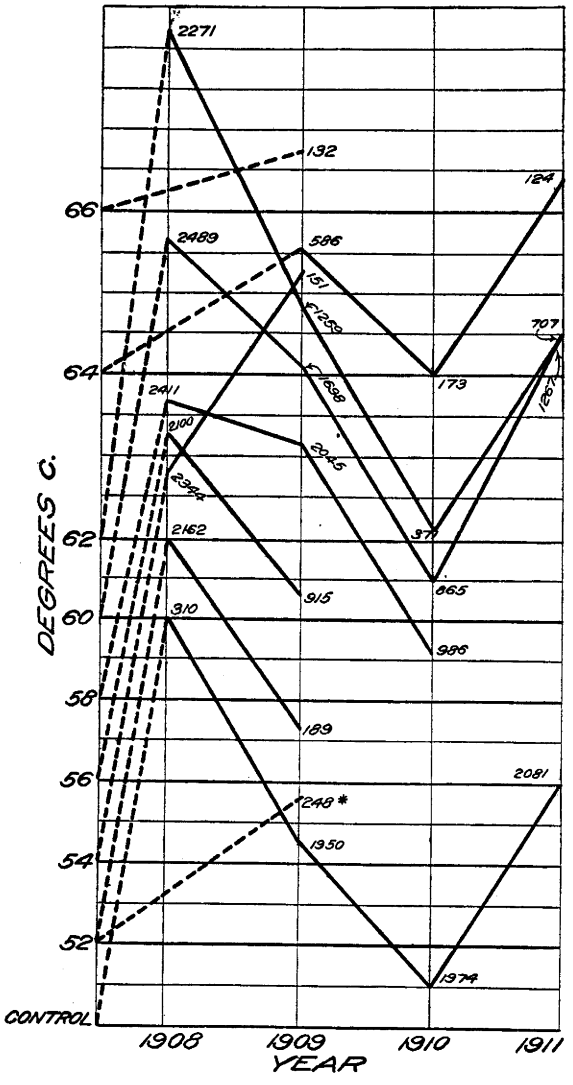

FIG. 6.—Curves summarizing for different years the percentages of infection in plantings of Red Amber sorgo after modified hot-water treatments of all durations of presoaking and treatment, but at various temperatures.

The inevitable conclusion from these experiments is that infection commonly takes place from some other source than seed-borne spores. This conclusion has been supported by tests of the effect of some of these treatments on the viability of the spores. Tables II and III present the results of these tests. They were somewhat obscured by the comparatively sparse germination so characteristic of these spores and by the development of the contaminations contained in the untreated spore material used in seeding check plates. The treatments with hot water were carried out, mostly on March 10, 1913, as follows.

Spores from Red Amber sorgo of the crop of 1911 were used in most cases. Before treatment they were thoroughly wet by shaking with distilled water. The dirt and foreign material were removed by centrifuging, and later the single spores were separated from the spore balls by the same method. In Table II, Nos. 1 to 14 and 29 to 34, inclusive, separated spores were used, while spore balls were used for the other treatments, except the last two, which were mixed. With a wire loop the spores or spore balls were transferred from the wet mass at the bottom of the centrifuge tube to tubes of water, which were then placed in the thermal bath. At the end of the period of treatment a portion of the spores in suspension was poured or pipetted out of the tube into melted agar at 43° C., in which they were shaken up and were then poured into a Petri dish. This portion was incubated at 27° to 28° C. and was examined from time to time under the microscope for germinating spores.

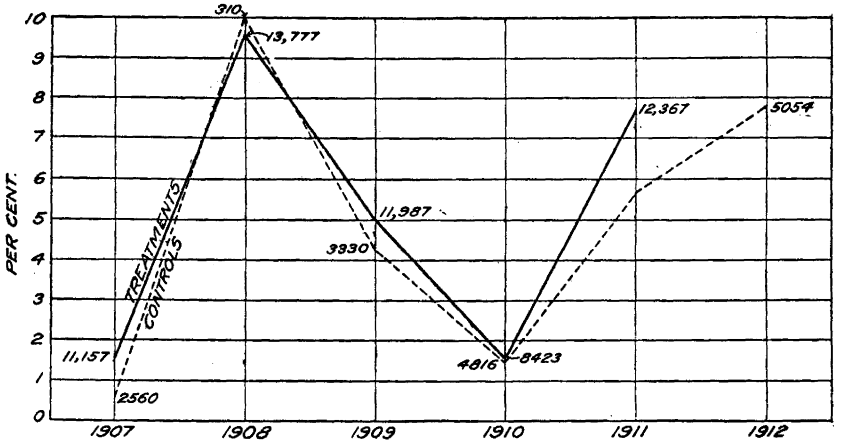

FIG. 7.—Curves summarizing for different years the percentages of infection: First, in plantings of sorgo after all seed treatments; and, second, in control plantings.

In the later treatments at 60° C. (Table II, Nos. 29 to 34, inclusive) the spores were subjected to the hot-water bath in the tubes of melted agar, thus avoiding the subsequent transfer. The first method would appear to give more chance for error, and to this is due, perhaps, the slight survival noted after rather severe treatments.

In Table II it is seen that moist heat is fatal within the upper range of temperatures used in the seed treatments (see fig. 6), and even dry heat seems injurious to the spores of this smut (Table II, Nos. 35 and 36). The plantings from hot-water and modified hot-water treatments of the seed showed a field infection in no way correlated with the thermal death point of the spores. About 24,000 plants grown from seed treated according to the latter method showed an infection of 5.9 per cent as against 3.1 per cent in about 3,500 plants grown from untreated seed. Over 15,000 of the plants from treated seed were of the Red Amber sorgo variety, which showed 6.5 per cent of smutted plants as against 4.2 per cent in the controls.

TABLE II.—*Results showing the effect of various hot-water and modified hot-water treatments on the viability of the spores of the head-smut organism*

Serial No.	Treatment.			Duration of test.	Number counted.	Germination.
	Duration.	Temperature.	Duration of presoaking.			
	Min.	*° C.*	*Hours.*	*Days.*		*Per cent.*
1	.....	Control.	(a)	2	1,225	3.8
				3	535	4.5
2	10	55	(a)	3	.....	Trace.
				4	.....	Trace.
3	20	55	(a)	3	1,500	0
				4	.....	0
4	10	60	(a)	3	3,000	.1
				5	.....	No increase.
5	20	60	(a)	3	3,000	0
				5	.....	0
6	10	65	(a)	4	2,500	.04
7	20	65	(a)	4	5,000	0
8	.....	Control.	6	2	1,046	9.6
				2	600	0
9	10	55	6	3	.....	1—
				4	400	3
				2	.....	0
10	20	55	6	3	.....	Trace.
				4	700	2.9
11	10	60	8	3	3,000	.03
				5	.....	.5—
12	20	60	8	3	4,000	0
				5	5,000	.02
13	10	65	8½	4	3,000	.33
14	20	65	8½	4	5,000	.04
				2	572	1.6
15	.....	Control.	(a)	3	.....	5+
				4	.....	No increase.
16	10	55	(a)	3	400	0
				4	.....	Trace.
17	20	55	(a)	3	500	0
				4	.....	0
18	10	60	(a)	3	550	0
				5	.....	0
19	20	60	(a)	3	.....	0
				5	.....	Trace.
20	10	65	(a)	4	.....	Trace.
21	20	65	(a)	4	.....	Trace.
22	.....	Control.	6	2	503	19.7
				2	402	Slight.
23	10	55	6	3	.....	2+
				4	100	30
				2	650	0
24	20	55	6	3	.....	0
				4	.....	0
25	10	60	8	3	.....	0
				5	.....	0
26	20	60	8	3	.....	0
				5	.....	0
27	10	65	8½	4	600	.17
28	20	65	8½	4	522	0
29	.....	Control.	(a)	2	1,000	4
				8	.....	No increase.
30	10	60	(a)	2	.....	0
				8	.....	0

[a] Not soaked.

TABLE II.—*Results showing the effect of various hot-water and modified hot-water treatments on the viability of the spores of the head-smut organism*—Continued

Serial No.	Treatment.			Duration of test.	Number counted.	Germination.
	Duration.	Temperature.	Duration of presoaking.			
	Min.	° *C.*	*Hours.*	*Days.*		*Per cent.*
31	20	60	(a)	2	.....	0
				8	.....	0
32	.....	Control.	6	2	950	4.5
				8	.....	No increase.
33	10	60	6	2	.....	0
				8	.....	0
34	20	60	6	2	.....	0
				8	.....	0
35		Control.	(a)	3	.....	13.1
36	5	70 Dry heat.	.....	2	.....	Slight.
				6	.....	Slight.

[a] Not soaked.

In the tests of the effect of fungicides on the spores the solutions of different strengths, including water for control, were prepared at a temperature of 22° to 23° C. and placed in culture tubes. The spore material was prepared as for the thermal tests and transferred to the tubes in the same way. The culture tubes were then thoroughly shaken. At the end of the period indicated in the tables the tubes were again agitated and with a pipette 5 c. c. were removed from each to the centrifuge tubes, which were immediately filled with water. The spores being thrown down by centrifuging, the water was poured off and the tubes refilled, this rinsing being repeated four or five times. The last rinsing water from the strongest treatment was poured on to the control, which was then recentrifuged, to make certain that the rinsing had removed the treating solutions effectively. Further water being added, enough of the suspension of spores was poured into a tube of melted carrot agar at about 43° C. to make a thickly seeded plate. The plate was poured immediately, incubated, and examined as in the other tests.

In the work with copper sulphate, solutions equivalent to from 0.35 per cent to 2.52 per cent of $CuSO_4$ were used in treatments of sorgo seed, some of which had had the glumes removed before treatment. In one series (1907) a 17-hour soak with the weakest of these solutions gave plants with 2.3 per cent infection as against none in the controls, while in another series (1911), using seed without glumes, a 10-minute treatment with the strongest solution resulted in 13.1 per cent of infected plants as against 2.8 per cent in the controls.[1] Other treat-

[1] The fact that all of these treatment experiments, except the modified hot-water treatments, were also infected by *Sphacelotheca sorghi* seems to have had a peculiar bearing on these comparative percentages. In nearly all cases a considerably larger amount of head smut occurred in the treated lots than in the controls, which, not having been treated, were heavily infected by the kernel smut. The latter seemed to get the start of the head smut and prevent its development, for no case of evident double infection, as was observed by Busse (1904, p. 381), was found. Thus, in the various treatments of Red Amber sorgo carried out in 1911 with formalin, cresol, copper sulphate, and hot water, 24 treated lots containing 3,616 plants averaged 10 per cent of head-smut and 2.6 per cent of kernel-smut infection, while 15 lots (3,081 plants), untreated or unsuccessfully treated for the kernel smut, contained 5.8 per cent of head smut as against an infection of 29 per cent by the kernel smut. One lot with 62.3 per cent of kernel smut had 3.3 per cent of head smut; in another the percentages were 57.1 and 1.8, respectively. This phenomenon seems to have an adequate explanation in the comparatively late period of infection shown for the head smut (see p. 365).

ments involving, with controls, some 3,500 plants, were equally ineffective and inconsistent in their results.

But the spores are not at all injured by even more severe treatments. Table III, Nos. 11 to 18, inclusive, gives the results of these tests performed with the spores on March 7, 1913. It might even be said that the development of conidia proceeded better in the plates containing treated spores, probably on account of the absence of contaminations, these being for the most part killed by the treatment. It is possible that a longer treatment, even with less concentrated solutions, would have killed the spores (Herzberg, 1895, p. 30), but this would be likely to injure the seed as well.

TABLE III.—*Results showing the effect of various formalin and copper-sulphate treatments on the viability of the spores of the head-smut organism*

Serial No.	Treatment.		Duration of test.	Number counted.	Germination.
	Duration.	Method.			
	Min.		*Days.*		*Per cent.*
1	.....	Control, not treated....	3	681	4.1
			7	.....	No increase.
2	34	0.16 per cent formaldehyde solution	3	.....	0
			7	1,000	1.6
3	33	0.24 per cent formaldehyde solution	3	.....	0
			7	.....	0
4	60	0.16 per cent formaldehyde solution	3	.....	0
			7	.....	0
5	60	0.24 per cent formaldehyde solution	3	.....	0
			7	.....	0
6	.....	Control, not treated....	3	541	13.1
			7	.....	No increase.
7	60	0.16 per cent formaldehyde solution	3	.....	0
			7	.....	Trace(?).
8	.....	Control, not treated....	3	.....	Slight.
			7	.....	Slight.
9	60	0.16 per cent formaldehyde solution	3	.....	0
			7	.....	0
10	60	0.24 per cent formaldehyde solution	3	.....	0
			7	.....	0
11	.....	Control, soaked 30 minutes	28	1,068	5.2
12	30	0.76 per cent copper-sulphate[a] solution...	28	1,044	5.0
13	30	1.52 per cent copper-sulphate solution....	28	1,034	5.2
14	30	2.52 per cent copper-sulphate solution....	28	1,000	4.0
15	.....	Control, soaked 60 minutes	28	1,034	4.5
16	60	0.76 per cent copper-sulphate solution....	28	1,000	1.8
17	60	1.52 per cent copper-sulphate solution....	28	1,000	5.0
18	60	2.52 per cent copper-sulphate solution....	28	1,000	6.0

[a] Copper sulphate = $CuSO_4$.

In an additional test intended to discover the influence of a residual effect of the fungicide after treatment without rinsing, it was found that the presence of a trace of copper sulphate in the medium does not hinder germination. However, Moore and Kellerman (1904, p. 29) found that the toxic action of dilute watery solutions of copper is overcome by certain substances present in most culture media; and Hawkins (1913, p. 68–75) has recently shown that soluble calcium and potassium salts also neutralize the toxicity of copper. The probability that some of these substances were present in the vegetable medium used makes the above test of residual action inconclusive. Nevertheless, Dandeno (1908, p. 60) states that *Ustilago zeae* germinates readily in a N/2,048 watery solution of copper sulphate. Copper fungicides do not appear to have a very penetrating action, and the sulphate certainly is not destructive to the head-smut spores within a limited time at ordinary temperatures.

McAlpine (1910, p. 298) found that a 0.12 per cent solution of formaldehyde did not affect the spores inside of 15 minutes. However, the formaldehyde treatment, when sufficiently severe, does kill them, as is shown in Nos. 1 to 10, inclusive, of Table III. These tests were with separate spores, except in the last three, in which spore balls were used.

In spite of this evidence that the spores do not survive one hour's treatment with a 0.16 per cent formaldehyde solution, it was found that seed given this and more severe treatments produced plants with 4.2 per cent of infection in about 3,000 plants (the estimates in the early experiments were by heads) which survived, as against 3.4 per cent in about 2,000 plants from untreated seed. The formalin treatment, therefore, is ineffective, but not because of failure to destroy external seed infection; and it may be said that this is true of the other chemical treatments of the seed, all of which have proved equally ineffective in prevention, even though, like copper sulphate, they may have had no lethal effect upon the spores. Indeed, plants from treated seed seemed the more easily infected in some instances.

FLORAL INOCULATIONS

The evident systemic character of the disease, however, immediately suggested the possible analogy with the loose smuts of barley and wheat. Kellerman's inoculations were made before the possibility of intraseminal infection was realized, and the question occurs, was not Jensen's (1888, p. 61) mistake, in assuming extraseminal infection to have taken place in the case of *Ustilago tritici* when a diseased wheat plant appeared among those he had inoculated, here repeated in the case of sorghum? While the loose character of the spore body and the echinulate spores of the head smut gave added force to the hypothesis of a floral infection, the abundant production of conidia and, as compared with the loose smuts (Appel and Riehm, 1911, p. 363), the prolonged viability of the spores, did not support this analogy.

Numerous floral inoculations, undertaken early in these investigations, also failed to give results supporting this view. These were carried out in several different seasons and at various stages of development in the ovary. Dry spores of the head smut were placed in a paper bag and shaken into the flowers by inverting the bag over a head and shaking thoroughly; sometimes they were placed inside the glumes with a camel's-hair brush. Some of the spores were germinated before applying them, and were sprayed into the flowers with an atomizer either by opening the glumes with forceps, or in the early morning while the plant was still in bloom; some of the heads were not covered, but some were kept covered for a time with paper bags or with a large lamp chimney to keep them moist. This was an extremely difficult matter, however, owing to the high winds and to the consequent rapid rate of evaporation, which, from an open water surface, often exceeds half an inch in 24 hours at Amarillo. While there was occasionally a rather high percentage of infection in the resulting plants, this was not the uniform result of any particular method of inoculaton; nor was it sufficiently large to obviate its explanation by infection of the plants during development in the field, in view of the fact that it did not occur consistently.

ENVIRONMENTAL EXPERIMENTS

In addition to the negative results of inoculations, it was found that seed from the same lots when planted at various points in the United States, or in different seasons at Amarillo, gave very different amounts of infection in the plants produced, while in plants from different lots of seed, grown at the same station, no consistent differences could be observed.

A preliminary experiment was carried out in 1908. The plants were all grown from the same lot of seed, yet those grown at Amarillo were 7.7 per cent smutted and those at Chillicothe, Tex., were 2 per cent smutted, while those at McPherson, Kans., were not affected at all. In 1910 a new series was begun. Plantings were made from two lots of seed at eight different stations, including Amarillo and Chillicothe, Tex., St. Paul, Minn., and Arlington, Va. Of these two lots, that from Chillicothe happened to develop the greater percentage of smutted plants at Amarillo, and the seed grown from it was therefore used for the plantings in 1911. In this and subsequent seasons the intention was to plant at each station seed from each of the places concerned and to use only seed descended from the original lot and grown in consecutive seasons at the same station. This was usually done, but, owing to various mishaps, the plantings at the four stations named were the only ones which were carried completely through the experiment as intended. The data from these four stations thus form a complete series and are summarized in Table IV. They involved in each case from about 150 to 800 plants; usually about 300.

TABLE IV.—*Summary of results showing the influence of locality on the occurrence of head smut*

Seed from—	Percentage of infection at—											
	Amarillo, Tex.			Chillicothe, Tex.			St. Paul, Minn.			Arlington, Va.		
	1910	1911	1912	1910	1911	1912	1910	1911	1912	1910	1911	1912
Amarillo, Tex.	1.6	14.71	10.86	0.14	0	7.34	0	0	0	0	0	0
Chillicothe, Tex.	3	13.09	12.29	.25	0	6.93	0	0	0	0	0	0
St. Paul, Minn.	.....	17.14	1.72	.....	.87	3.52	.....	0	0	.....	0	0
Arlington, Va.	.....	7.56	2.9	.....	2.6	4.46	.....	0	0	.....	0	0

From this it may be seen that no infection occurred at Arlington or at St. Paul. Only a trace of it has ever occurred at St. Paul, except in inoculated plants in 1912. It has not been present at all at the Arlington Experimental Farm or in its immediate vicinity during the three years in question, so far as the writer was able to discover by careful examination. Yet seed from St. Paul produced the highest percentage recorded at Amarillo in 1911, although showing no infection at either Arlington or St. Paul in that year; and seed from Arlington has always produced some smutted plants at the two Texas points. Of the four seed lots used in 1911, the Arlington seed produced the largest number of infected plants at Chillicothe. Moreover, seed grown at either of the two Texas stations never produced smutted plants when grown at the other two stations, although inoculated plants showed abundant infection at St. Paul in 1912 (see Table V, plat E). It should be noted, too, that seed from the same lots used for the Amarillo plantings in 1910 and 1911 were planted at Amarillo in the ensuing years and produced infected plants as follows: 1910 lots, replanted in 1911, 3.8 per cent and 15.6 per cent, respectively; 1911 lots, replanted in 1912, 1.8, 2.7, 0, and 1.8 per cent, respectively. These figures are evidently in no way comparable or consistent with those of the year before, as shown in Table IV.

EXPERIMENTS WITH PROTECTED SEED

As may have been already observed, particularly in connection with the slight irregularities of the curves in figure 6 (see footnote, p. 351), positive conclusions from comparative amounts of infection in small lots of plants at Amarillo are not warranted without consistent results from oft-repeated experiments. However, the appearance of any infection in plants from seed protected from contamination gives additional evidence that the infection is not carried with the seed.

Thus, 177 plants were grown at Amarillo in 1912 from seed produced in the greenhouse at Washington, D. C., on heads which had been covered with transparent paper bags from before flowering until they were thrashed out by hand. One plant (0.6 per cent) was infected. Similarly, 1,669 plants grown in 1912 from seed of 18 heads protected in the

same way but produced in the field at Amarillo in 1911 showed 6.4 per cent of infection. The high winds had torn some of the bags at times, but they were replaced as soon as possible. Moreover, four of them remained intact throughout; yet of the 206 plants grown from the resulting seed, 13, or 6.3 per cent, were infected. This was scarcely less than the average natural field infection in 1912. (See fig. 7.)

This evidence is a particularly strong negation of the floral infection theory, especially when it is noted that the seed lot from the greenhouse in Washington, D. C., produced 8 infected plants out of 18 when the seedlings were artificially inoculated. (See Table V, plat C, No. 5.)

INFECTION EXPERIMENTS

It has been made clear by the results already described that floral infection is not involved in the life history of this parasite and that seed-borne spores, though doubtless functioning at times in distributing the fungus from one district to another, by no means constitute the determining factor in the general field infection. The apparent contradiction in the evidence so far presented—one which has led to many confusing surmises and recommendations in the literature of the subject—remains to be explained by positive evidence of infection from artificial inoculations.

A series of inoculation experiments carried on at Amarillo, Tex., in 1911, duplicated at Amarillo, Tex., at St. Paul, Minn., and at Manhattan, Kans., during the season of 1912, and twice repeated in the greenhouse at Washington, D. C., has confirmed these observations and demonstrated that the presence of the parasite in the soil about the growing seedling is productive of successful infection under any of the conditions prevailing in these various habitats. These results are presented in Table V.

EXPLANATION OF TABLE V

In tabulating these results considerable abbreviation has seemed desirable, and it is herewith explained. When special reference to this explanation is necessary, the abbreviations in Table V are inclosed in parentheses. Under each of the following main headings the column with the same heading in the table is explained.

"Date."—The date given in the column provided is the date of inoculation except in a few cases, usually controls, when it is inclosed in parentheses and indicates the date of planting.

"Seed Lot."—Five different lots of seed, all of the variety Red Amber sorgo (S. P. I. No. 17548), were used and are indicated, in the column provided, by the following symbols:

"I." From the crop of 1910 at Amarillo, Tex. When in parentheses, as "(I)," the seed had the glumes still inclosing it; otherwise it was without them, having been separated in water from the seed which had retained the glumes through the thrashing process.

"II." Seed without glumes (separated in water, as in I); from the crop of 1911 at Amarillo. This seed was treated with a 0.24 per cent formaldehyde solution for one hour, except where the symbol is in parentheses "(II)."

"III." Seed from a head grown at Amarillo in 1911, which had been kept covered with a transparent paper bag from before flowering until thrashed out by hand. The parentheses simply indicate a different head as the source of seed.

"IV." Seed from heads grown in the greenhouse at Washington, D. C., during the winter of 1911–12 and kept covered, as above, from before flowering until thrashed out by hand.

"V." Seed without glumes (separated in water, as in I); from the crop of 1911 at Akron, Colo. Treated with 0.16 per cent formaldehyde solution for 10 minutes after a thorough washing.

"Spore Lot."—The mixed lot of spores used is so indicated; the other five lots, all collected from Red Amber sorgo at Amarillo, are indicated as follows:

"*A*." Collected in the fall of 1910.

"*B*." Collected in September, 1911. The parentheses indicate conidia from cultures first isolated from single spores of this lot (see p. 341) in February, 1912.

"*C*." Collected in the fall of 1912.

"Method."—The methods used in making inoculations are classified—

First, as to the condition of the host plant when inoculated (or planted, in the controls):

"a"=dry seed;

"b"=germinating seed;

"c"=older plants.

Second, as to the character of the inoculating material:

"m"=dry spores;

"n"=suspension of spores in which a few were germinating;

"p"=conidia.

Third, as to the general procedure in inoculating:

"x"=heavy application of a mass of the inoculating material, usually so as to completely cover the seed or seedling when planting it, or, on older plants, to cover the inoculated part;

"y"=lighter application—dusting of dry spores before planting or spray of material in water;

"z"=inoculation of the plat by raking smutted heads into the soil after plowing in the spring. "zz" in plat C, No. 7=inoculation two years in succession, the same plat being used as for plat A, No. 11, the year before.

Fourth, the controls, which were not artificially inoculated, are indicated in this column.

Fifth, special methods in inoculation are indicated by parentheses, as follows:

"bm(x)" in plat A, No. 7=the soil in the opened row was heavily inoculated at planting;

"bn(x)" in plat B, Nos. 1 and 2, plat D, No. 1, and plat E, No. 1, and

"bp(x)" in plat C, Nos. 1 and 2, plat D, No. 2, and plat E, No. 2=both seedling and soil were inoculated;

"cm(x)" in plat C, No. 8, plat D, No. 8, and plat E, No. 5=the spores were placed about the root crown just beneath the surface of the soil;

"cp(x)" in plat E, No. 6=the conidia were taken from carrot-agar culture and smeared on the base of the plant with a flat inoculating needle;

"bm(y)," "bn(y)," and "b(control)" in plat E, Nos. 8, 9, and 10=the ground was thoroughly wet down both before and after planting, the seed only being inoculated;

"bn(y)" in plat A, Nos. 1 and 2=the seed only was inoculated;

"bn(y)" in plat A, Nos. 3 and 4=the soil only in the opened row was inoculated;

"cp(y)" in plat C, No. 9, and plat D, No. 9=conidia were sprayed on the root crown, which was then re-covered with moist earth.

TABLE V.—*Results showing infection produced in Red Amber sorgo by extraseminal inoculations*

PLAT A[a]

[Planted at Amarillo, Tex., in the field; counted Sept. 12, 1911.]

Serial No.	Date.	Seed lot.	Spore lot.	Method.	Total number of plants.	Infection.
						Per cent.
1	May 25	I	Mixed	bn(y)	325	4.9
2	do	(I)	do	bn(y)	383	3.7
3	do	I	do	bn(y)	103	5.8
4	do	(I)	do	bn(y)	70	5.7
5	do	I	do	bmy	165	6.7
6	do	(I)	do	bmy	210	5.3
7	do	I	do	bm(x)	130	34.6
8	May 26	(I)	do	bm(x)	106	23.6
9	May 25	I	do	amy	200	10
10	do	(I)	do	amy	292	4.1
11	(May 23)	I	*A*	az	34	11.7
12	do	(I)	*A*	az	110	5.5
13	May 25	I	..	a, control[b]	196	6.6
14	do	(I)	..	a, control	127	12.6
15	do	I	..	a, control	272	7.3
16	do	(I)	..	a, control	444	3.8

PLAT B[a]

[Planted at Washington, D. C., in pots in the greenhouse of the Department of Agriculture. The even numbers were planted in a 2-inch top dressing of clean sand, while the other pots (odd numbers) contained only potting soil; counted May 16, 1912.]

1	(c)	II	*B*	bn(x)	8	50.0
2	(c)	II	*B*	bn(x)	7	14.3
3[d]	(c)	II	*B*	bmx	3	100
4	(c)	II	*B*	bmx	3	100
5	(c)	II	..	a, control	5	0
6	(c)	II	..	a, control	7	0
7	(c)	(II)	..	a, control	7	0
8[d]	(c)	(II)	..	a, control	8	0
9	(e)	II	*B*	bmx	2	50
10	(e)	II	*B*	bmx	1	100

PLAT C[f]

[Planted at Amarillo, Tex., in the field; counted Sept. 7, 1912.]

1	May 28	II	(*B*)	bp(x)0.6 to 1.2[g]	41	26.8
2	May 30	III	(*B*)	bp(x)	5	0
3	May 28	II	*B*	bmx 0.6 to 1.2[g]	45	66.6
4	May 30	(III)	*B*	bmx	1	0
5	May 28	IV	*B*	bmx	18	44.4
6	do	II	*B*	amx	102	42.2
7	(May 29)	II	*B*	azz	522	21.45

[a] Inoculations by the author.
[b] Treated for one hour with 0.24 per cent formaldehyde solution.
[c] About Nov. 15, being the date of planting in Nos. 5, 6, 7, and 8.
[d] See Plate XXXV, figure 3.
[e] About Feb. 8.
[f] Inoculations performed by Mr. E. C. Johnson.
[g] The numbers given indicate in centimeters the length of the plumules in Nos. 1 and 3, and the average height of the plants in Nos. 8 and 9. In the latter case the plants were mostly unbranched as yet. In Nos. 10 and 11 the plants were younger than in No. 3.

TABLE V.—*Results showing infection produced in Red Amber sorgo by extraseminal inoculations*—Continued

PLAT C—Continued

Serial No.	Date.	Seed lot.	Spore lot.	Method.	Total number of plants.	Infection.
						Per cent.
8	June 25	II	*B*	cm(x)5[a]	136	2.94
9	do	II	(*B*)	cp(y)5[a]	112	7.1
10	May 29	II	*B*	bmx[a]	45	42.2
11	do	II	*B*[b]	bmx[a]	68	39.7
12	(May 29)	II	..	b, control	112	32.1
13	do	II	..	a, control	148	1.4
14	do	II	..	a, control	185	3.2
15	do	(II)	..	a, control	136	25
16	do	(II)	..	a, control	114	3.5
17	(May 25)	(III)	..	a, control	51	3.9
18	do	III	..	a, control	50	4
19	(May 29)	II	..	a, control[c]	328	1.5
20	do	(II)	..	a, control[c]	268	0

PLAT D[d]

[Planted at Manhattan, Kans., in the field; counted Aug. 30, 1912.]

1	June 4	II	*B*	bn(x)2[e]	1	0
2	do	II	(*B*)	bp(x)2[e]	24	0
3	June 3	II	(*B*)	bp(x)5[e]	1	0
4	June 4	II	*B*	bmx2[e]	24	29.1
5	June 3	II	*B*	bmx3[e]	3	0
6	do	II	*B*	bmx5[e]	1	100
7	June 3–4	II	*B*	amx	50	10
8	June 4	II	*B*	cm(x)	200	0
9	do	II	(*B*)	cp(y)[c]	75	0
10	(June 4)	II	..	a, control	210	0
11	do	II	..	a, control[c]	731	0
12	do	II	..	b6, control[e]	5	0
13	do	II	..	b2, control[e]	26	0

PLAT E[f]

[Planted at St. Paul, Minn., in the field; counted about Sept. 20, 1912.]

1	June 7	II	*B*	bn (x)	49	10.2
2	do	II	(*B*)	bp (x)	(*g*)	0
3	do	II	*B*	bmx	30	26.7
4	do	II	*B*	amx	49	36.7
5	July 5	II	*B*	cm (x)[h]	(*g*)	0
6	do	II	(*B*)	cp (x)	(*g*)	0
7	June 8	II	..	a, control	(*g*)	0
8	June 11	II	*B*	bm (y) 2.5[h]	85	24.7
9	do	II	*B*	bn (y) 2.5[h]	98	2
10	(June 11)	II	..	b2.5 (control)[h]	(*g*)	0

[a] The numbers given indicate in centimeters the length of the plumules in Nos. 1 and 3, and the average height of the plants in Nos. 8 and 9. In the latter case the plants were mostly unbranched as yet. In Nos. 10 and 11 the plant were younger than in No. 3.

[b] Kept outside in cloth bag through the winter at Amarillo.

[c] Planted apart from the rest to avoid contamination from inoculated rows.

[d] Inoculations by the author, assisted by Dr. N. E. Stevens.

[e] These numbers indicate the time, in days, between setting the seed to germinate and inoculating and planting it.

[f] Inoculations performed by Dr. E. M. Freeman and Mr. J. H. Parker.

[g] Plants not counted.

[h] This number indicates the approximate length of the plumules in centimeters at the time of inoculation and planting.

TABLE V.—*Results showing infection produced in Red Amber sorgo by extraseminal inoculations*—Continued

PLAT F [a]

[Planted at Washington, D. C., in the greenhouse in beds separated by partitions 1 foot deep in the soil; counted Apr. 3, 1913.]

Serial No.	Date.	Seed lot.	Spore lot.	Method.	Total number of plants.	Infection.
						Per cent.
1	Nov. 1–9	V	*B* and *C*	Various [b]	67	55.2
2	do	V	..	a, control	16	0
2 [c]	do	V	..	do	16	31.25

[a] Inoculations by the author.

[b] These inoculations were performed with various methods and stages of growth in an effort to get more exact information. With the small number of plants, necessitated by the use of a greenhouse, differences in the amount of infection appearing were of little significance in view of the impossibility of properly controlling conditions. Most of the plants were not directly watered, except at planting (nor were they, in the control), until mature in the spring. Although all were grown in separate beds instead of pots and obtained ample moisture from below, they were much stunted by greenhouse conditions.

[c] The same plants as above, but counted Oct. 3, 1913.

While most of the results of these inoculations are positive beyond a doubt, an important negative result, as yet unexplained, should be pointed out. The conidia, in spite of the care taken to be certain of their identity(see p. 341), have never produced the slightest evidence of infective power in the few trials made in the field (plat C, Nos. 1, 2, and 9; plat D, Nos. 2, 3, and 9; and plat E, Nos. 2 and 6). Brefeld (1895, p. 30) has found that oat smut, like many other pathogenic organisms, loses its virulence after several months in artificial cultures. Unless *Sorosporium reilianum*, as cultivated on carrot agar in these investigations, lost its infective power very quickly, however, this explanation does not seem adequate, for new cultures grown artificially for only two or three weeks produced no infection when inoculated on 15 plants at the same time and under the same conditions as plat F, No. 1. The conidia have not been observed to produce infection threads as figured by Brefeld (1883, pl. 11, fig. 7).

The first question which arises on considering the fact, here now clearly shown, that extraseminal infection does take place, is, What factor has been introduced to bring about successful infection when so many former attempts had failed? The results given in Table V, while not exhaustive, do make clear several of the essential points in the parasite's life history which will at least partially answer this question. The method designated under the abbreviation bmx will be observed to have produced the most consistently positive results wherever tried. Except at Amarillo, the only other methods which produced over 20 per cent infection were bn(x) in pots in the greenhouses of the Department (plat B, Nos. 1 and 2 of Table V), amx at St. Paul (plat E, No. 4), and bm(y) at St. Paul (plat E, No. 8), besides the inoculations later attempted in the greenhouse (plat F).

Since none of these methods can be presumed to correspond closely to the natural process of infection, the conclusions drawn from them must be largely a matter of inference. The small number of plants and the abnormal conditions in the greenhouse make it unnecessary to consider method bn(x), in plat B, Nos. 1 and 2, further than to note that both seeds and soil were heavily inoculated and that the seeds were germinating. Moreover, method bmx in the same series (plat B, Nos. 3 and 4) produced 100 per cent of infection on six plants, so that both these methods appear to have been proportionately more successful than elsewhere, probably because of the more thorough technique where so few plants were concerned. It appears, indeed, that the abundance of infectious material provided has been the most salient factor involved. Without it at Amarillo natural infections were often so numerous that the effect of inoculation was not perceptible; compare, for instance, plat A, No. 7, with plat A, Nos. 9 and 13, and plat A, No. 8, with plat A, Nos. 10 and 14. Method amx, which is closely similar to bmx on account of the large amount of spore material provided, the seedling having to grow up through the latter in both cases, has also produced a comparatively large percentage of infection, even exceeding bmx (plat E, No. 3) at St. Paul.

These results immediately suggest that no such crucial period for infection of the seedling obtains in the case of this smut as has been observed by Brefeld (1895, p. 46) for *Ustilago cruenta*, for the presence of the infecting organism during the whole of the early development of the host produces the disease when its presence on the seed alone will rarely do so. While *U. cruenta* was not able to infect, in Brefeld's experiments, after the leaf sheath had been split as far down as 1 cm. from the tip, the plumules of the plants inoculated by method bm(y)—a dusting of dry spores over the seedlings—in plat E, No. 8, averaged close to 2.5 cm. in length and yet were nearly as abundantly infected as those which were smaller and more heavily inoculated four days before (plat E, No. 3).

The difference between the latter and plat E, No. 4 (method amx) is not sufficient to militate against the conclusion that a late period of infection is possible, although it has seemed from the character of the infection in the mature plant, as revealed by the histological studies already discussed, that the infection in the field at Amarillo is usually quite early in its origin. That it is systemic in the individual culm more characteristically than in the plant as a whole, however, supports this idea of late infection (see p. 348). Investigation has shown, moreover, that the hyphæ were at least not widely disseminated in the growing tissues of several seedlings which later developed infection. In the seasons of 1910[1] and 1911 about 200 seedlings at three to four weeks after planting were dissected and a part of the meristem—that containing the primary

[1] The dissections in this season were made by Mr. V. L. Cory.

growing point—was removed and preserved. The plants were then induced to produce a second growth from what remained. The meristem of those which developed head smut at maturity was then carefully examined; yet in none of the 16 plants which developed the disease could the hyphæ be found in the parts preserved.

In additon to the negative evidence of these dissections, Mr. Karl F. Kellerman, of the Bureau of Plant Industry, stated to the writer in recent conversation that he performed a number of experiments with this smut by artificial inoculations on sorghum in the greenhouse while working in Ohio with his father, Dr. W. A. Kellerman. The plants were in pots and were inoculated at stages varying from the time they first appeared above ground until they were about 5 inches high. The method used was to wash the soil away from the roots, sift dry spores over them, and re-cover with soil. While some indications pointed to infection through the roots, this was not definitely established. Whatever the mode of entry, however, the parasite proved able under the conditions in the greenhouse to infect plants at all the stages at which they were inoculated.

In the recent greenhouse experiments (Table V, plat F, No. 1) some of the plants were successfully inoculated after the first leaf had begun to turn green. But, most unexpected of all, after leaving these plants to grow all summer it was found in October that the control (plat F, No. 2) contained five smutted plants, whereas the original culms which developed in April showed no sign of the disease. Other plants, too, which had not been smutted in the spring had grown smutted culms by fall. While Hecke (1907, p. 572) has presented similar facts as proof of shoot or branch ("Trieb") infection by *Ustilago antherarum*, in the case of sorghum, at least, there is some uncertainty as to the exact point of infection. The inoculation of the nodal buds has been tried a few times in the greenhouse without result. This does not preclude the possibility of such an infection, however, and more careful work supported by histological observations is needed.

It does not seem that the spread of the disease from plant to plant under greenhouse conditions makes it probable that such an occurrence is at all common in the field, but it does add certainty to the conclusion that infection by this smut is by no means confined to the early seedling stage of the host. This, then, together with the sparse germination of the spores, readily explains the repeated failures to produce any appreciable amount of infection by inoculation of the seed.

In Table V, plats C, D, and E, it will be observed that the same lot of seed, "Seed lot II," previously treated with a 0.24 per cent solution of formaldehyde, was used for nearly all the inoculations. This seed produced plants free from head smut at both Manhattan, Kans., and St. Paul, Minn. (plats D, Nos. 10, 11, 12, and 13, and E, Nos. 7 and 10), except when artificially inoculated; but at Amarillo all but one of the

control plantings (plat C, Nos. 12 to 20, inclusive) were infected—two of them to the extent of 25 per cent or more—while the percentage of infection in the successful inoculations was not remarkably greater, as compared with controls, than was produced by the same methods at the other two stations. It is thus indicated that at Amarillo, or wherever this smut occurs at all commonly, the parasite is present, doubtless in the soil, in much the same way as the common maize smut, *Ustilago zeae*, is present where maize is much grown.

PREVENTION OF HEAD SMUT

Since the period of infection appears to be quite indefinite, the prevention of this disease seems almost as difficult a problem as that of dealing with common maize smut, and, where prevalent, is a more serious question on account of the more systematic character of the infection. This latter fact, however, suggests a possible, though very doubtful and as yet untried, specific measure for prevention—i. e., the treatment of the soil about the seed at planting time in some such way as is done for onion smut—in the hope of keeping infection away from such buds as develop early in the life of the plant.

The fact that the disease occurs most abundantly in a district where manures or fertilizers have rarely, if ever, been used obviates the explanation of its occurrence on this basis. The Panhandle of Texas is, however, a region of high winds favorable to its spread, and the cutting out and burning of the whole plant when one is found infected should, of course, be recommended. Rotations planned to avoid continuous cropping of the particularly susceptible sorgo varieties on the same ground or to the leeward of prevailing winds from such a field should also considerably reduce the amount of head smut.

An important element in the relation of the problem to the grain-sorghum grower is the fact that milo, as has been noted by Freeman and Umberger (1908), is a variety apparently immune from all the sorghum smuts. This crop is widely grown in the southern part of the Great Plains, and it should be possible, theoretically, to obtain various immune varieties adapted to other sections by breeding from it. Since the cause of this immunity is not yet apparent, however, it can not be definitely stated that its hybrids will partake of this character. Kafir and broom corn, while much less susceptible to this smut than the sorgos, are quite subject to the attack of the kernel smut. This lack of immunity might prove serious to these crops or even to maize, should the head smut ever become as abundant as has maize smut (*Ustilago zeae*) in many sections. The latter is indigenous to America, however, and since the head smut is not, it may be hoped that adequate quarantine measures would prevent its spread and lead, perhaps, to its final eradication.

SUMMARY

(1) The head smut of sorghum, *Sorosporium reilianum* (Kühn) McAlpine, was first reported from Egypt in 1868. It has been found to be a destructive parasite, though not yet of widespread occurrence in this country. It occurs also on maize, or Indian corn.

(2) The organism has been grown in artificial culture. Its growth is almost exclusively conidial under favorable conditions, the optimum temperature being 28° to 30° C. As with several others of the Ustilagineae, spore-like bodies are occasionally found in older cultures.

(3) Although perfect sori of the parasite are not usually produced in every head of a plant, most of the stools and branches are so affected, even when producing no spore-bearing tissue, that the inflorescence is sterile and often peculiarly proliferated. This vegetative stimulus results also in the development of the lateral buds into branches.

(4) Histological studies indicate an early period of infection and the systemic nature of the disease. The lateral buds carry the infection in their meristematic tissue apparently from the time of their formation when the culm is starting to differentiate the nodes.

(5) The work of other investigators, though not conclusive, pointed to infection from seed-borne spores and the possibility of applying the usual seed-treatment methods for preventing the disease. Both of these contentions have been shown to be untenable by an extensive series of ecological experiments and exhaustive tests of various sterilizing agents, including the use of thermal methods, on the seed.

(6) Numerous floral inoculations failed to show that the infection was produced intraseminally and carried over in the seed to the next crop. On the other hand large percentages of infection were repeatedly produced by inoculation of the seedlings with dry spore material, some becoming infected in the greenhouse even after the first leaf had emerged from the sheath and begun to turn green. While the process of infection has not yet been observed histologically, it is clearly proved that the parasite is not carried with the seed, but is wind-distributed in the locality in which it occurs, doubtless infecting the seedling from the soil.

(7) Though widely distributed in the tropical and semitropical countries of the world, the head smut has been known in this country for only about 35 years. Methods of combating it are especially needed in order to prevent its spread. Fortunately the widely grown variety, milo, has proved immune from all the smuts of sorghum.

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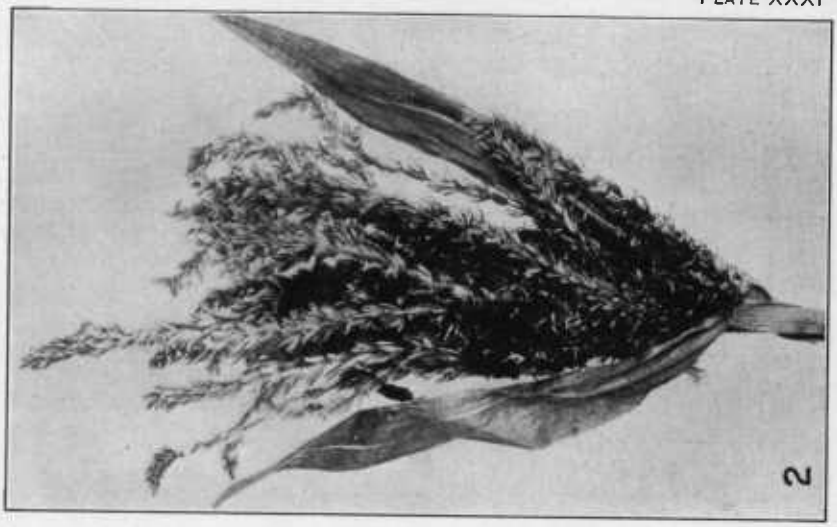
PLATE XXXI

Fig. 1.—Head smut in ear of maize (after McAlpine).
Fig. 2.—Head smut in tassel of maize (after Evans).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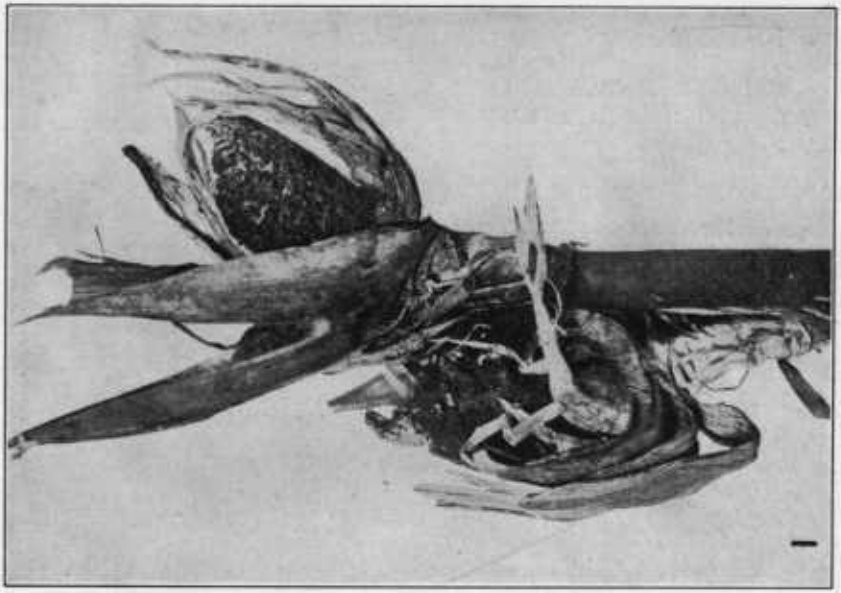

1


a
b
c
1


2

PLATE XXXII

Fig. 1.—The three American species of sorghum smut on Blackhull kafir: (a) *Sphacelotheca cruenta*, (b) *Sorosporium reilianum*, (c) *Sphacelotheca sorghi*. Photographed by author.

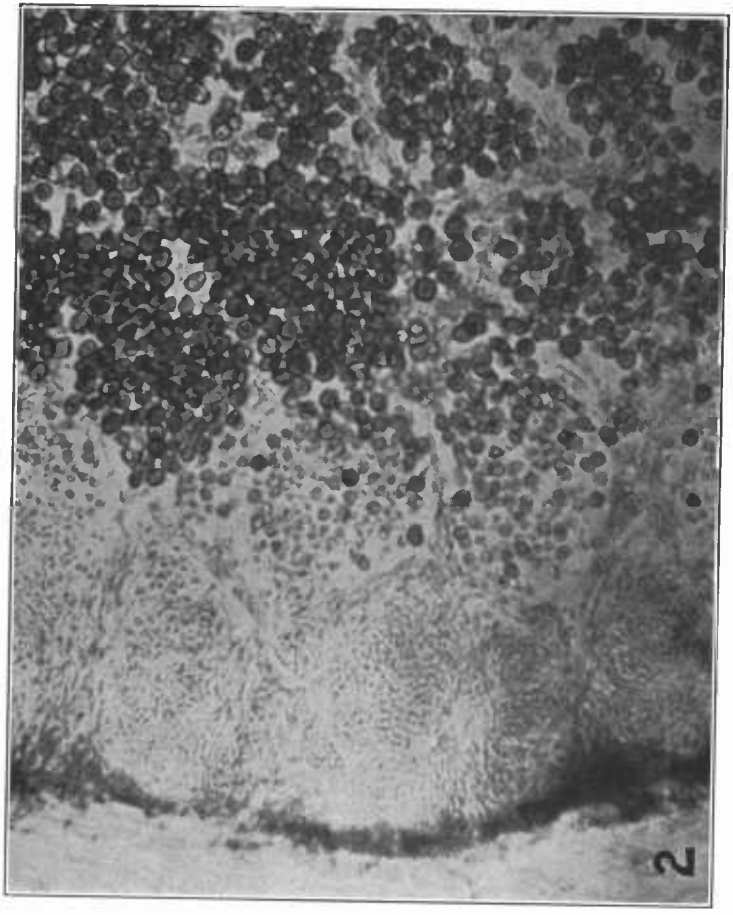
Fig. 2.—Head smut, *Sorosporium reilianum* (Kühn) McAlp., on "sumac" sorgo, San Antonio, Tex., October, 1913. Photographed by Mr. Karl F. Kellerman.

51131°—14——4

PLATE XXXIII

Fig. 1.—Section through young sorus, showing hyphal aggregates preceding spore formation. × 710. Photomicrographed by author.

Fig. 2.—Section through immature sorus. Note the fibrovascular bundle on the left, about which the spores, none of which were as yet quite mature, were developing in groups even in the earliest stages. × 365. Photomicrographed by author.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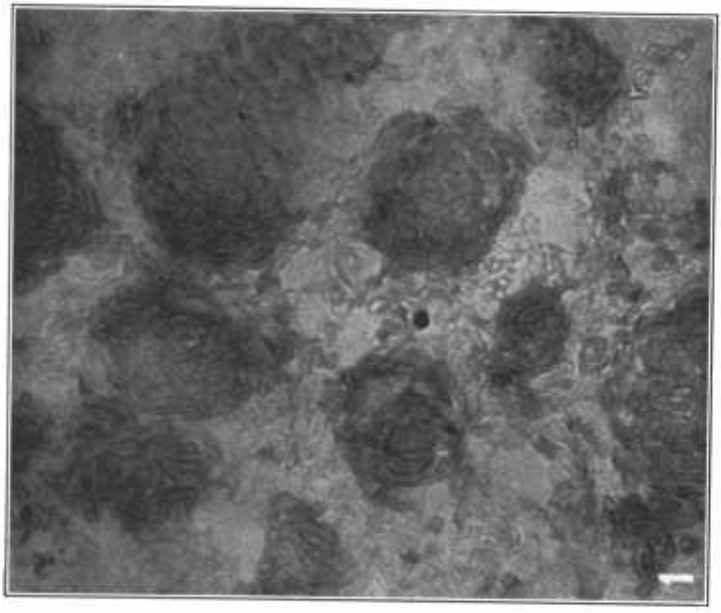
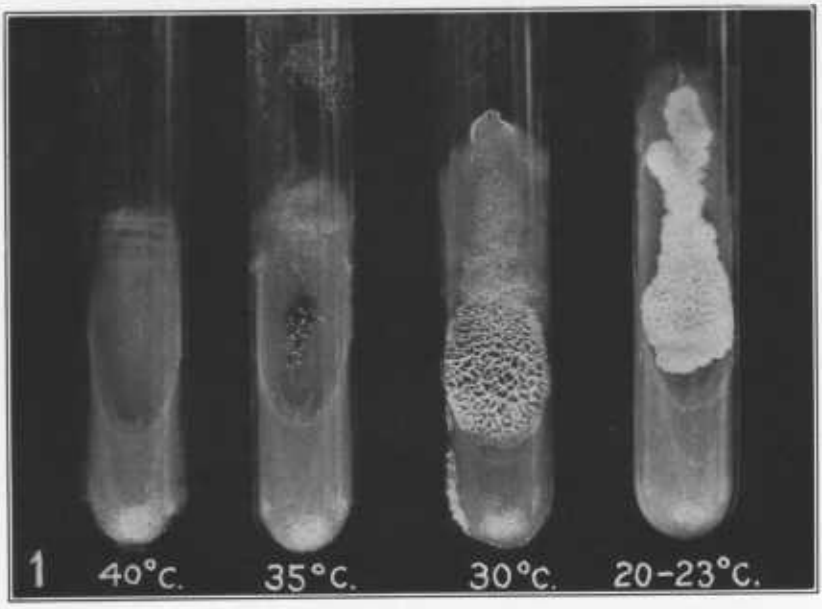
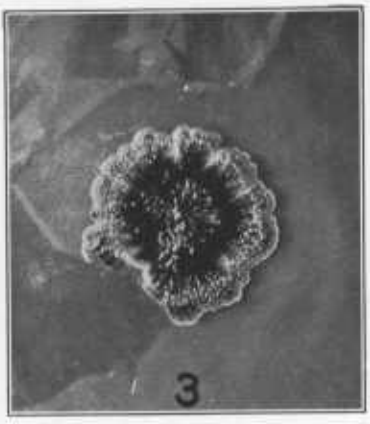



1
40°C.
35°C.
30°C.
20-23°C.


2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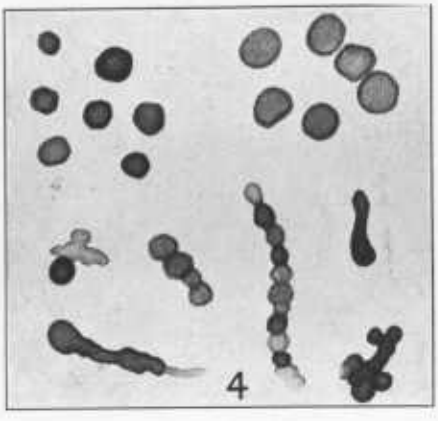
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PLATE XXXIV

Fig. 1.—Growth of organism of head-smut of sorghum and maize on carrot agar at various temperatures; cultures about 6 weeks old. × 4/5.

Figs. 2 and 3.—Twenty-two days' growth of organism of head-smut of sorghum and maize on synthetic glucose agar (fig. 2) and on carrot agar (fig. 3). Photographed by Mr. E. C. Johnson and author.

Fig. 4.—Chlamydospores of organism of head-smut of sorghum and maize formed in culture in peptonized maltose solution. In the upper right-hand corner are shown some natural spores for comparison. × 450. Drawn by author.

PLATE XXXV

Fig. 1.—Smutted culms of Amber sorgo, showing the characteristic sterility of the main panicle. Photographed by Mr. E. C. Johnson.

Fig. 2.—Proliferated head of Blackhull kaoliang, with one normal and one smutted head. Photographed by author.

Fig. 3.—Smutted and nonsmutted plants of Red Amber sorgo used in head-smut infection experiment. Control pot (see Table V, plat B, No. 8) on left; inoculated pot (Table V, plat B, No. 3) on right, showing three smutted plants. Photographed by Mr. E. C. Johnson.


1
2
3

PLATE XXXVI

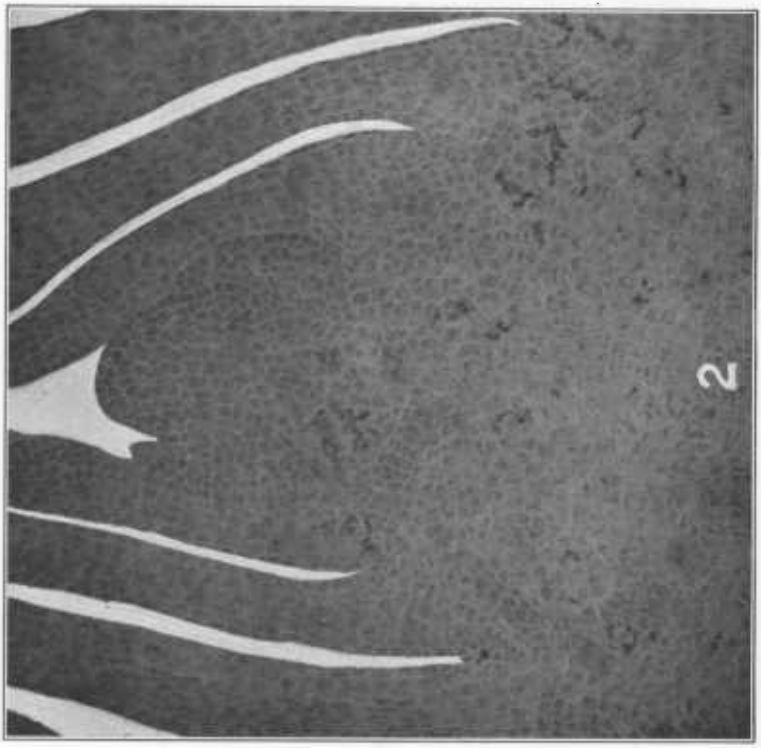
Panicular formation in apex of proliferated sorghum flower. Longitudinal section, showing presence of hyphæ of head smut. × 70. Photomicrographed by Mr. W. W. Gilbert and author.

PLATE XXXVII

Longitudinal sections through the growing points of two of the buds indicated in text figure 1, showing hyphæ of the head smut. × 150.

Fig. 1.—Bud 3 of culm B1. Positions of hyphæ are shown in text figure 2.

Fig. 2.—Bud 7 of culm N2. Photomicrographed by Mr. W. W. Gilbert and author.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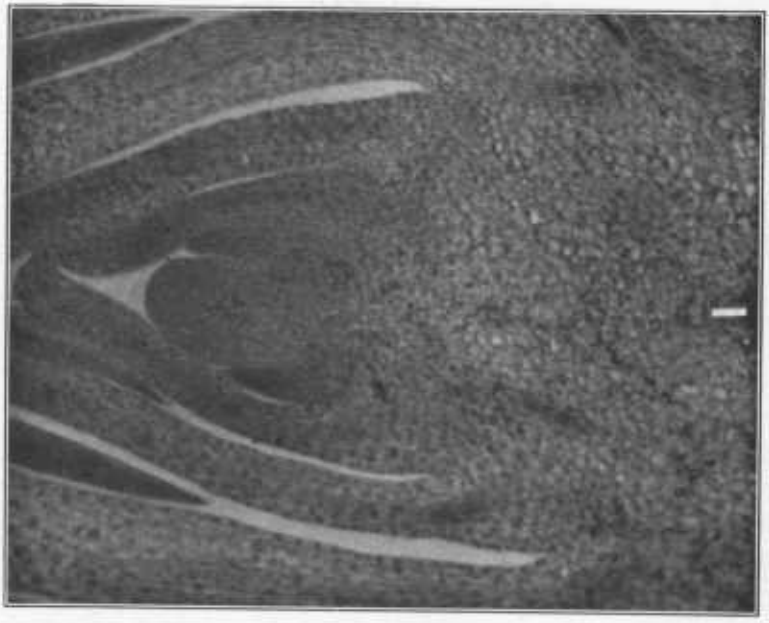
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